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Novel use Of Hydroxyurea in an African Region with Malaria (NOHARM): a trial for children with sickle cell anemia

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Key Points:

1. Compared to placebo, hydroxyurea did not increase the incidence or severity of malaria events in Ugandan children with sickle cell anemia
2. Hydroxyurea provided significant clinical and laboratory benefits, suggesting it will be safe and effective across sub-Saharan Africa

ABSTRACT

Hydroxyurea treatment is recommended for children with sickle cell anemia (SCA) living in high-resource malaria-free regions, but its safety and efficacy in malaria-endemic sub-Saharan Africa, where the greatest sickle cell burden exists, remain unknown. *In vitro* studies suggest hydroxyurea could increase malaria severity, and hydroxyurea-associated neutropenia could worsen infections. NOHARM was a randomized, double-blinded, placebo-controlled trial conducted in malaria-endemic Uganda, comparing hydroxyurea to placebo at 20 ± 2.5 mg/kg/day for 12 months. The primary outcome was incidence of clinical malaria. Secondary outcomes included SCA-related adverse events, clinical and laboratory effects, and hematological toxicities. Children received either hydroxyurea (N=104) or placebo (N=103). Malaria incidence did not differ between children on hydroxyurea [0.05 episodes/child/year, 95% CI (0.02, 0.13)] versus placebo [0.07 episodes/child/year (0.03, 0.16)]; the hydroxyurea/placebo malaria incidence rate ratio was 0.7 [(0.2, 2.7), p=0.61]. Time to infection also did not differ significantly between treatment arms. A composite SCA-related clinical outcome (vaso-occlusive painful crisis, dactylitis, acute chest syndrome, splenic sequestration, or blood transfusion) was less frequent with hydroxyurea (45%) than placebo (69%, p=0.001). Children receiving hydroxyurea had significantly increased hemoglobin concentration and fetal hemoglobin, with decreased leukocytes and reticulocytes. Serious adverse events, sepsis episodes, and dose-limiting toxicities were similar between treatment arms. Three deaths occurred (two hydroxyurea, one placebo, none from malaria). Hydroxyurea treatment appears safe for children with SCA living in malaria-endemic sub-Saharan Africa, without increased

severe malaria, infections, or adverse events. Hydroxyurea provides SCA-related laboratory and clinical efficacy, but optimal dosing and monitoring regimens for Africa remain undefined.

INTRODUCTION

Sickle cell anemia (SCA) is a life-threatening hematological disorder and among the world's most prevalent hereditary diseases, with over 300,000 affected babies born each year.[1] The vast majority of these births occur in sub-Saharan Africa, where an estimated 50-90% of children with SCA will die by age five years, often without an established diagnosis.[2] The heterozygous sickle gene mutation confers a strong survival advantage against malaria,[3] which explains why the allele frequency is highest in malaria-endemic regions of Africa.[1]

Hydroxyurea has proven laboratory and clinical efficacy for both children and adults with SCA.[4-7] Its mechanisms of action are multiple and incompletely understood, but fetal hemoglobin (HbF) induction in erythroid cells is critical for the inhibition of intracellular sickling. Treatment also has salutary effects on blood cell adhesion, morphology, and rheology.[8] Further, hydroxyurea is a safe drug for SCA, with low incidence of treatment-related toxicity and no serious long-term effects observed to date. Hydroxyurea is currently approved by the US Food and Drug Administration for adults with severe symptoms and by the European Medicines Agency for affected adults and children above age two years. Based on a large and compelling body of evidence accumulated over the past 30 years, evidence-based guidelines published by the National Heart, Lung and Blood Institute of the National Institutes of Health

strongly recommend wider usage, including offering treatment to infants as young as nine months of age.[9]

The benefits of hydroxyurea treatment for children with SCA would be greatest in countries within sub-Saharan Africa, if hydroxyurea were found to be safe and have its predicted efficacy in these high-burden areas. However, the effects of hydroxyurea on the presentation and clinical course of malaria must also be considered. Hydroxyurea may directly affect several factors related to the pathogenesis of severe malaria, with potentially deleterious consequences. Some *in vitro* and animal studies suggest that hydroxyurea increases endothelial intracellular adhesion molecule-1 (ICAM-1) expression,[10] which could enhance parasite adhesion to endothelium,[11] and also increases TNF-α levels,[12] both of which are associated with increased malaria severity and death.[13] Other studies have challenged these findings,[14,15] but definitive human data are lacking. If hydroxyurea induces effects that shift the clinical course in SCA from uncomplicated to severe malaria, this could increase mortality despite benefits for the underlying SCA.[16] In addition, in low-resource settings, hydroxyurea may cause neutropenia that could lead to increased severity of the many bacterial infections commonly observed in African children with SCA.[17]

In contrast to these adverse effects, it is possible that hydroxyurea could have beneficial effects against malaria since HbF, which is increased by hydroxyurea, inhibits *Plasmodium falciparum* growth *in vitro*.[18] Hydroxyurea treatment also generates nitric oxide,[19] which protects against severe malaria in animals[20,21] and humans.[22] With these contrasting potential mechanisms, the risks and benefits of hydroxyurea in a malaria endemic setting remain

unknown, and many critical questions remain regarding the safety, feasibility, and efficacy of hydroxyurea for children with SCA living in malaria-endemic settings within sub-Saharan Africa.

For these reasons, we conducted a prospective randomized controlled double-blinded placebo-controlled clinical trial, the Novel use Of Hydroxyurea in an African Region with Malaria (NOHARM), in young Ugandan children with SCA, to determine the safety and efficacy of hydroxyurea in a malaria-endemic region. NOHARM is registered on ClinicalTrials.gov as NCT01976416.

METHODS

Study design. A detailed description of the study site, design, and procedures was previously published.[23] Briefly, NOHARM was a randomized double-blinded placebo-controlled clinical trial. Due to concerns about the risks of severe malaria and infection with hydroxyurea treatment, the consensus among local experts and the local institutional review board supported a placebo-controlled trial, despite the expected SCA-related treatment benefits in children with SCA. It was agreed, however, that study participants should be given the opportunity to receive subsequent open-label hydroxyurea, if no danger signal was observed from the blinded study treatment. NOHARM was conducted at Mulago Hospital Sickle Cell Clinic (MHSCC) in Kampala, Uganda. Kampala has seasonal malaria transmission, with high outpatient and inpatient burdens. Protocol approval was obtained from the institutional review boards of the Makerere University School of Medicine, Indiana University, University

of Minnesota, and Cincinnati Children's Hospital, as well as the Uganda National Drug Authority and Uganda National Council for Science and Technology.

Study participants. Children receiving care at MHSCC were eligible if 1.00-3.99 years of age and living within 50km of the clinic. Study inclusion criteria included confirmed SCA, weight ≥5.0kg, and willingness to comply with study procedures. Children with severe malnutrition or known chronic medical conditions, current hydroxyurea treatment, or blood transfusion in the previous 30 days were excluded. Parents of the study participants gave written informed consent.

Randomization and masking. Children meeting inclusion criteria completed enrolment and after screening, were randomized 1:1 to hydroxyurea or placebo by the Data Coordinating Center (DCC) at Cincinnati Children's Hospital as described.[23] Study allocations were randomly assigned by the statistician, using computer-generated sequences in block files of 8 participants. Study treatments were supplied with A or B labels, and the clinical coordinating center staff, study pharmacists, families and caregivers, and all but two members of the DCC were masked to treatment allocation.

Medications. Study treatment (Addmedica, Paris) included oral hydroxyurea (Siklos®) as 1000mg scored tablets and 100mg dispersible tablets, or placebo tablets of identical size and appearance. Treatment was administered once daily at 20 ± 2.5 mg/kg for 12 months, with dose adjustments in both arms for weight gain and hematological toxicities.[23]

Procedures. All participants received standard care for SCA including folic acid, penicillin prophylaxis, and pneumococcal vaccination. For malaria prophylaxis, children received

insecticide-treated mosquito nets and monthly sulphadoxine-pyrimethamine. Study participants were seen for the randomization visit (Month 0), and for scheduled visits at 2 weeks post treatment initiation, monthly from months 1-4, and at months 6, 8, 10 and 12 for a total of ten scheduled visits with monitoring for clinical malaria and toxicities. Caregivers were instructed to return to clinic or hospital whenever the child was unwell; all febrile children received malaria testing. After completing the blinded treatment phase, participants could receive open-label hydroxyurea, as per local Ethics Committee recommendations.

Measurements. Complete blood counts with leukocyte differential and absolute reticulocyte count (ARC) were measured at each visit (Sysmex Corporation, Kobe, Japan) plus blood chemistries using a Cobas 6000 analyzer Model C501 (Roche Diagnostics, Indianapolis, USA). HbF was quantified by capillary electrophoresis (Minicap, Sebia, Paris France). Malaria was assessed by peripheral blood smear using Giemsa staining: each slide was read by two certified microscopists, with a third reading to resolve any discrepancies.

Clinical definitions. All children with measured fever (axillary temperature ≥37.5C) in the clinic or fever by history were tested for malaria by microscopy. Children with measured fever or a history of fever and detectable *Plasmodium* species infection of any density on blood smear were diagnosed with clinical malaria, as is standard in this area. Malaria was treated with parenteral artesunate followed by oral artemether-lumefantrine, if the child was hospitalized, or oral artemether-lumefantrine, if the child was not hospitalized. The diagnosis of sickle-related clinical events followed published definitions,[24] with modifications such as pneumonia based on clinical rather than X-ray findings. Clinical sepsis was defined as

presentation with fever and an unwell appearance, for which intravenous antibiotics were given. Full definitions for clinical adverse events are provided in Table 1.

Study outcomes. The primary study outcome was incidence of clinical malaria. Secondary outcomes included: (1) a composite of one or more SCA-related adverse events (pain, dactylitis, acute chest syndrome, splenic sequestration, or transfusion); (2) clinical adverse events; and (3) dose-limiting toxicities.

Statistical analysis. We estimated malaria incidence would be between 0.3 and 1.3 malaria episodes per year, based on a study based in Kampala that includes community children (lowest incidence estimate) and children with severe malaria (highest incidence estimate) (CC John, unpublished data). With these rates of malaria incidence, and variance to mean ratio 1.7, we estimated that 100 children per treatment arm would provide 90% power (alpha = 0.05) to detect between 59% and 34% difference in incidence between treatment arms, respectively. Study data were entered into OnCore and analyzed using R (Vienna, Austria, version 3.2.4). The primary analysis included all randomized participants per intention-to-treat, with $p<0.05$ considered significant. Interim analysis for safety was performed after 100 children completed blinded study treatment. The difference in malaria incidence rate between the hydroxyurea and placebo groups was analyzed using negative binomial regression. For secondary outcomes, $p<0.01$ was considered significant. Continuous measures are described as Mean (SD), and treatment arms were compared using the two-sample Welch's t-test, or when within the same individuals using a paired Welch's t-test. Categorical measures are described using percent or frequency, and compared using the

Pearson χ^2 test with Yates correction or Fisher's exact test. Malaria episodes are reported as mean per child (95% CI). Time to malaria infection is described by Kaplan-Meier curves. Groups were compared for cumulative incidence (i.e., time to infection) using Gray's method, with all-cause death treated as a competing event.[25]

RESULTS

A total of 213 children were enrolled between September 24, 2014 and October 2, 2015; among these, 208 were randomized to either hydroxyurea or placebo (Figure 1). One child was later deemed ineligible due to an elevated baseline ALT, so that child's data were removed from the analyzed dataset. The treatment arms had similar baseline demographic, laboratory, and clinical measures (Table 2). No child had symptomatic or asymptomatic *Plasmodium* species parasitemia at enrollment.

Malaria occurred at a low rate throughout the study, perhaps reflecting excellent adherence to malaria prophylaxis. Over the one year study period, malaria testing was done for 235 episodes of measured fever or history of fever in study participants, with only 12 fever episodes due to malaria. Malaria incidence did not differ between children prescribed hydroxyurea [0.05 episodes per child per year, 95% CI (0.02, 0.13)] versus placebo [0.07 episodes per child per year (0.03, 0.16)]; the hydroxyurea/placebo malaria incidence rate ratio was 0.7 [(0.2, 2.7), p=0.61]. Time to infection also did not differ significantly between hydroxyurea and placebo (Figure 2). All 12 malaria episodes were due to *P. falciparum*; one child had co-infection with *P. malariae*. Three children on hydroxyurea had a total of five

malaria episodes, compared to seven children on placebo with a total of seven malaria episodes (Table 3). The median parasite density was 26,410 parasites/µL (minimum, 1,064; maximum, 193,705). Six of the 12 episodes of malaria were severe, requiring hospitalization (two with hemoglobin concentration <5 g/dL, one with impaired consciousness, one with hemoglobin concentration <5 g/dL and impaired consciousness, and two unable to take oral medication). All ten study participants with malaria recovered. In the 12 confirmed cases of malaria, there were 4 episodes with concomitant clinical AE: Vaso-occlusive pain crisis (N=3) and ACS/pneumonia (N = 1). In addition, there were 2 episodes of malaria with concomitant SAE: splenic sequestration (N=1) and bacteremia (N=1).

A per-protocol composite clinical outcome included one or more SCA-related clinical events (vaso-occlusive painful crisis, dactylitis, acute chest syndrome/pneumonia, splenic sequestration, or blood transfusion). The proportion of children with this outcome was significantly lower in the hydroxyurea arm than the placebo arm (45% versus 69%, Table 3, p=0.001). For individual clinical events, vaso-occlusive pain and hospitalizations were significantly less frequent with hydroxyurea than placebo (Table 3, Figure 3). This difference in hospitalization rate was largely driven by differences in vaso-occlusive crises, which accounted for 42% of all hospitalizations. The number needed to treat to prevent one hospitalization was 6.4, while the number needed to treat to prevent a SCA-related event was 2.5. No strokes occurred in either treatment arm during the one-year treatment period. Clinical sepsis was more frequent with placebo (12.6%) than hydroxyurea (5.8%), but this difference was not statistically significant (p=0.14). Blood cultures were positive in only two episodes, both for *Staphylococcus aureus,* one in each treatment arm.

Serious adverse events, defined as death, an acute life-threatening event, or hospitalization for more than seven days, occurred equally with six in each treatment arm (Table 3). During the blinded treatment phase, three children died (two hydroxyurea, one placebo) and five others withdrew from the study. Causes of death were presumed sepsis (one hydroxyurea, one placebo) and sudden death, cause unknown (hydroxyurea). In two of the SAE listed in Table 3, two participants had malaria concomitant with bacteremia or splenic sequestration, as noted above.

The two treatment arms had similar counts of laboratory adverse events except anemia (Table 3). Specifically, low hemoglobin (<6.0 g/dL) occurred more frequently in children receiving placebo than hydroxyurea, while the frequencies of neutropenia, thrombocytopenia and reticulocytopenia did not differ significantly between treatment arms. Similarly, the frequency of protocol-defined dose-limiting hematological toxicities did not differ between treatment arms (Table 3). Neutropenia was notably rare, occurring only once in only two participants over the entire study.

Children receiving hydroxyurea had significant treatment-associated increases in hemoglobin concentration, mean corpuscular volume (MCV), and HbF compared to those receiving placebo (Table 4). Substantial increases in HbF were observed for both children below and above the median enrollment age, despite starting with different baseline HbF levels. Conversely, children on hydroxyurea had significant decreases in white blood cell count, absolute neutrophil count, absolute reticulocyte count, and platelets compared to children receiving placebo (Table 4). The treatment arms did not differ in changes in alanine transferase

or creatinine. Medication adherence, assessed by careful questioning at each clinic visit, was deemed excellent in both treatment arms.

DISCUSSION

In this prospective randomized double-blinded placebo-controlled trial of young children with SCA living in Uganda, hydroxyurea therapy was both safe and efficacious. The two treatment arms did not differ in the incidence, severity, or other outcomes from malaria infection; in the incidence of clinical sepsis or bacteremia; or in the number of laboratory adverse events or dose-limiting toxicities, including neutropenia. In contrast, the previously described laboratory and clinical benefits of hydroxyurea therapy were clearly observed in this young population. The increases in hemoglobin concentration and HbF levels, along with decreases in neutrophils and reticulocytes, were similar to values observed in US-based trials,[4-7,26] while the rates of important clinical SCA-related events such as vaso-occlusive painful crisis and hospitalizations were similarly reduced. Taken together, these data suggest that hydroxyurea should be strongly considered as an important therapeutic option for young children with SCA living in malaria endemic areas.

NOHARM represents the first randomized trial of hydroxyurea in Africa. The study design captured all possible malaria events, with blood smear testing for all episodes of current and recent fever. The incidence of malaria in our cohort was low, suggesting effective protection by insecticide-treated bed nets and monthly oral malaria prophylaxis with sulfadoxine-pyrimethamine provided to all study participants, or possibly a decreased risk of

clinical malaria in children with SCA. Some form of malaria prophylaxis for children with SCA is a standard recommendation in most African countries,[27] and insecticide-treated bed net use for children <5 years of age in malaria endemic areas of Africa has increased dramatically over the past decade, to an average of >60% coverage,[28] so the preventive measures used in this study are similar to those in many other malaria endemic areas where children with SCA live. However, adherence to these recommendations is highly variable, so our study findings may not apply to children with SCA not on malaria prophylaxis and/or not using insecticide-treated bed nets. Since malaria incidence was low in our study, the range of relative differences in malaria incidence between hydroxyurea and placebo is broad (95% CI for incidence rate ratio 0.2 to 2.7), but the absolute differences in episodes is small (95% CI 2-13 episodes/100 children/year with hydroxyurea versus 3-16 episodes/100 children/year with placebo). Hydroxyurea did not worsen malaria severity, since hospitalizations for malaria did not differ between treatment arms, and no child died of malaria. Because of the low malaria incidence, this study does not provide definitive guidance regarding the safety of hydroxyurea in all malaria endemic areas, but the lack of increased risks of malaria incidence, severity, and outcome is reassuring. The incidence of malaria in these children with SCA further suggests that absolute differences in malaria risk with hydroxyurea will be very small. However, risks of hydroxyurea may differ in areas of higher malaria transmission, and genetic or environmental factors in other areas could affect risk of hydroxyurea toxicity or malaria-hydroxyurea interactions. In light of the present study data showing efficacy of hydroxyurea against pain crises and hospitalizations, with no evidence of increased malaria or infection risk in this area of low malaria transmission, it may be difficult to justify additional placebo-controlled trials in

areas of higher malaria transmission. If additional placebo-controlled studies are not conducted, future studies of open-label hydroxyurea in other malaria endemic regions should carefully document the rates and complications of malaria during hydroxyurea treatment. These data will help determine the long-term safety profile of hydroxyurea for children living in malaria-endemic regions.

All five of the study children hospitalized with severe malaria (one child was hospitalized twice) survived, in contrast to an earlier study from Kenya in which four of five children with SCA hospitalized for severe malaria died (80% mortality).[16] The presence of malaria parasites on peripheral blood smear also did not increase the risk of death during hospitalization, contrasting with a Tanzanian study of children with SCA, which reported increased mortality in hospitalized children with parasitemia.[29] In contrast, another study from Kenya reported no deaths among 38 children with SCA and severe malarial anemia, the most common form of severe malaria affecting these children.[30] Similarly, in a recent study in Kampala conducted by our group, none of 22 children with SCA who developed severe malarial anemia died.[31] Together, these study findings cast doubt on the contention that severe malaria causes high rates of mortality in children with SCA. In the first two studies cited, which described increased mortality from malaria in children with SCA, malaria prophylaxis was either not given routinely[16] or consisted of chloroquine.[29] The sulfadoxine-pyrimethamine prophylaxis given in the present study is likely more effective than chloroquine prophylaxis. In addition, the two studies were conducted during a period of much lower bed net coverage, and a significant proportion of children in these earlier study cohorts received chloroquine or sulfadoxine-pyrimethamine for treatment of uncomplicated malaria, instead of the more effective current

standard of care, artemisinin-combination therapy. Together, these factors may have played a role in the higher mortality seen with malaria in these prior cohorts.

Increased infection in children with drug-induced neutropenia was an additional concern regarding hydroxyurea treatment in an area where invasive bacterial infection is common in children. However, neutropenia was rare in NOHARM and did not differ on hydroxyurea versus placebo treatment, and episodes of pneumonia, clinically defined sepsis, and bacteremia did not differ between hydroxyurea or placebo treatment arms. These data confirm findings observed in the US,[5] and should help allay safety concerns about hydroxyurea and infection risk related to neutropenia in low-resource settings.

Hydroxyurea was also associated with significantly fewer SCA-related clinical events, specifically vaso-occlusive crises, dactylitis, and hospitalizations. Other laboratory outcomes of long-term clinical importance for children with SCA, including increases in hemoglobin concentration and HbF, as well as decreases in leukocyte, neutrophil, and reticulocyte counts, were all more favorable in children receiving hydroxyurea than placebo, further supporting the drug's efficacy in this study population. Despite the differences in nutritional status from US-based populations, as evidenced by the low baseline Z-scores, the hydroxyurea responses were remarkably similar to published results in the BABY HUG trial for both clinical and laboratory effects.[5] The potential benefits of hydroxyurea treatment on growth in NOHARM are also important to assess, but have not yet undergone formal analysis.

After the double-blinded treatment phase, the entire NOHARM cohort was offered open-label hydroxyurea as per the study design, and all but one family opted for treatment.

Future analysis can thus compare the effects of early initiation and the longer-term effects of treatment. Since hydroxyurea escalated to maximum tolerated dose is standard in the US,[7,25] additional studies should assess how SCA-related adverse events and hematological responses compare for children receiving fixed-dose treatment versus dose escalation regimens. Outcomes were excellent in the present study with a safe and relatively easily administered fixed dose (20 mg/kg), so it will be important to investigate how treatment benefits and risks differ with different dosing schemes of hydroxyurea for children with SCA living in malaria-endemic areas. Children in African countries often have limited access to health care facilities and those facilities typically have limited lab testing capability. Less frequent laboratory monitoring would allow wider implementation of hydroxyurea treatment.

Additional studies that include longer duration of hydroxyurea in malaria-endemic areas will help determine optimal drug dosing, the range of adverse events, and malaria incidence in areas with higher malaria transmission. Until then, the present study documents that with adequate monitoring, mosquito nets, and malaria prophylaxis, fixed-dose hydroxyurea treatment for children with SCA in malaria-endemic areas is safe with no increased incidence of malaria, sepsis, bacteremia, or serious adverse events. In combination with the observed clinical efficacy (fewer painful events and hospitalizations), the NOHARM study findings support the wider use of hydroxyurea for children with SCA living in malaria-endemic regions across sub-Saharan Africa.

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AUTHORSHIP CONTRIBUTIONS

ROO, REW, and CCJ designed the study, supervised the trial, analyzed the results, and wrote the first draft of the manuscript. TSL helped coordinate many critical aspects of the trial to ensure its safe and successful operational execution. CMN, HAH, and PK enrolled patients, collected data, and helped interpret the results. AL and JSH performed statistical analyses for the trial. All authors participated in the editing of the manuscript and approved the final version.

DISCLOSURES OF CONFLICT OF INTEREST

Dr. Ware is a consultant for Global Blood Therapeutics and Nova Laboratories; is on an advisory board for Agios Pharmaceuticals; receives research support from Bristol Myers-Squibb; and serves on a Data and Safety Monitoring Board for the US Food and Drug Administration. None of these disclosures is relevant to the results and conclusions of the NOHARM trial.

Nothing to disclose: ROO, CMN, TSL, AL, HAH, PK, JSH, CJ

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Table 1. NOHARM Adverse Event Definitions.*

Clinical Adverse Event Terminology	**Definition**
Vaso-Occlusive Pain Crisis/Dactylitis	Vaso-occlusive pain crisis: acute pain and tenderness in an area of the body, with or without swelling, with no other diagnostic explanation. Dactylitis: Vaso-occlusive crisis (acute pain, tenderness, and swelling) localized to hands or feet.
Pneumonia/Acute Chest Syndrome	Pneumonia: history of fever or measured axillary temperature ≥37.5˚C, with tachypnea and cough. Acute chest syndrome: signs of pneumonia above plus chest pain and/or tenderness.
Clinical Sepsis	Measured fever and ill appearance, requiring IV antibiotics
Acute Splenic Sequestration	Increase in splenic size from last physical examination, accompanied by a decrease in hemoglobin of ≥2 g/dL
Upper Respiratory Infection	Child with general well appearance with rhinorrhea, nasal congestion or cough
Gastrointestinal-related	Diarrhea, vomiting, constipated, intestinal obstruction
Malaria	Measured fever (axillary temperature ≥37.5ºC) or fever by history and Plasmodium species infection on blood smear
Other infection	Other infections, diagnosed clinically
Other (e.g., injury)	Other diseases not included above diagnosed during visits for illness
Laboratory Adverse Events**	**Definition**
Anemia	Hemoglobin < 6 g/dL
Reticulocytopenia	Absolute reticulocyte count <80 x 10^9/L and hemoglobin < 7 g/dL
Neutropenia	Absolute neutrophil count <1.0 x 10^9/L
Thrombocytopenia	Platelet count <80 x 10^9/L
Elevated AST/ALT	AST>150 IU/L, ALT>150 IU/L
Elevated Bilirubin	Total bilirubin > 5 mg/dL

* The diagnosis of sickle-related clinical events followed published definitions,[24] with modifications such as pneumonia and clinical sepsis.
**Laboratory AE definitions represent the values necessary for a Grade 2 event.

Table 2. Baseline demographic, clinical, and laboratory characteristics of the NOHARM randomized cohort.

	Hydroxyurea (N=104)	Placebo (N=103)	P-Value
Demographics, N (%)			
Age at enrollment (years), mean (SD)	2.2 (0.9)	2.3 (0.9)	0.3148
Male	55 (53%)	57 (55%)	0.8298
Parent completing secondary school	32 (31%)	35 (34%)	0.7614
Running water in the home	14 (13%)	11 (11%)	0.6885
Growth Measures, Mean (SD)			
Height (cm)	85.6 (8.8)	86.3 (8.2)	0.5615
Weight (kg)	11.3 (2.1)	11.5 (2.1)	0.4604
Z-score (weight for length/height)	-0.40 (1.06)	-0.39 (1.06)	0.9316
Past Medical History, N (%)			
Dactylitis	80 (77%)	82 (80%)	0.7639
Vaso-Occlusive Crisis	88/103 (85%)	81/103 (79%)	0.3421
Stroke	0	0	-
Splenomegaly	6/93 (6%)	5/99 (5%)	0.7619^
Acute chest syndrome	21/103 (20%)	13/100 (13%)	0.2219
Transfusion	56 (54%)	57 (55%)	0.9393
Hospitalization within 1 year of enrollment	65 (63%)	54 (52%)	0.1851
Laboratory Measures, Mean (SD)			
Hemoglobin (g/dL)	7.5 (1.1)	7.6 (1.0)	0.5214
Mean corpuscular volume (MCV, fL)	79 (9)	80 (9)	0.8248
Fetal hemoglobin [(HbF)/(HbF + HbS), %)]	14.6 (7.1)	13.3 (6.0)	0.1591
Absolute reticulocyte count (ARC, x 10^9/L)	380 (122)	381 (112)	0.9623
White blood cell count (WBC, x 10^9/L)	19.0 (7.2)	18.7 (5.4)	0.7148
Absolute neutrophil count (ANC, x 10^9/L)	6.5 (3.1)	6.2 (2.6)	0.4023
Platelets (x 10^9/L)	358 (171)	416 (138)	0.0075
Alanine transferase (ALT, U/L)	18 (9)	19 (9)	0.5478
Creatinine (mg/dL)	0.28 (0.09)	0.28 (0.07)	0.6068

A total of 208 children were randomized to either hydroxyurea (N=104) or placebo (N=104). One participant was later deemed ineligible, and those data were removed from the dataset. Values are shown as the Mean (SD) or the number of study participants with the measure/total tested for that measure (% affected). ^Fisher exact test due to the low frequency.

Table 3. Adverse events in the NOHARM study population.

	Hydroxyurea (N=104)		Placebo (N=103)		p-value
	Events	Participants	Events	Participants	
Serious Adverse Events	6	6	6	6	
Bacteremia/Sepsis	2	2	2	2	1.0^
Acute Chest Syndrome/Pneumonia	1	1	2	2	0.62^
Vaso-Occlusive Crisis	0	0	1	1	0.50^
Acute Splenic Sequestration	2	2	0	0	0.50^
Anemia	0	0	1	1	0.50^
Sudden Death	1	1	0	0	1.0^
SCA-Related Events (Composite)					
Vaso-occlusive pain crisis, dactylitis, acute chest syndrome, splenic sequestration or blood transfusion		47		71	0.001
Clinical Adverse Events	232	76	308	88	
Vaso-Occlusive Pain Crisis/Dactylitis	58	38	106	59	0.004
Acute Chest Syndrome/Pneumonia	24	21	32	24	0.71
Clinical Sepsis	8	6	16	13	0.14
Acute Splenic Sequestration	0	0	0	0	-
Upper Respiratory Tract Infection	107	54	108	62	0.29
Gastrointestinal-related	15	13	15	12	1.0
Malaria	5	3	7	7	0.21^
Other infections	8	7	14	14	0.16
Others (e.g., injury)	7	7	10	9	0.78
Clinical Interventions	34	14	53	32	
Transfusion	14	12	18	17	0.41
Hospitalization	20	12	35	28	0.007
Laboratory Adverse Events	57	31	78	46	
Anemia	40	25	65	42	0.015
Reticulocytopenia	3	3	6	5	0.50^
Neutropenia	2	2	0	0	0.50^
Thrombocytopenia	12	11	4	4	0.11
Elevated AST/ALT	0	0	2	1	0.50^
Elevated Bilirubin	0	0	1	1	0.50^
Dose-Limiting Toxicities	21	15	17	13	
Anemia	6	4	8	8	0.36
Reticulocytopenia	1	1	5	5	0.12^
Neutropenia	2	2	0	0	0.50^
Thrombocytopenia	12	11	4	4	0.11

All serious adverse events (SAE), adverse events (AE), and laboratory adverse events (Lab AE) were compared between treatment arms. Proportions of affected participants were compared by Chi-squared tests with Yates correction. ^Fisher exact test instead of Chi-square, due to the low expected frequency. Full definitions of adverse events are provided in Table 1.

	Month 12			Change from baseline		
Laboratory Measures	**Hydroxyurea**	**Placebo**	**p-value**	**Hydroxyurea**	**Placebo**	**p-value**
Hemoglobin (g/dL)	8.7 (1.3)	7.4 (1.0)	<0.001	1.2 (1.2)	-0.1 (0.9)	<0.001
Mean corpuscular volume (MCV, fL)	88 (9)	81 (8)	<0.001	9 (7)	1 (5)	<0.001
Fetal hemoglobin [(HbF)/(HbF + HbS), %]	22.9 (8.6)	10.4 (4.8)	<0.001	8.5 (6.7)	-3.1 (3.3)	<0.001
Enrollment age below median	24.1 (8.5)	12.1 (4.8)	<0.001	7.9 (7.4)	-4.1 (3.5)	<0.001
Enrollment age above median	21.4 (8.7)	8.8 (4.2)	<0.001	9.3 (5.6)	-2.2 (2.8)	<0.001
Absolute reticulocyte count (ARC, x 10^9/L)	247 (107)	391 (122)	<0.001	-144 (119)	11 (119)	<0.001
White blood cell count (WBC, x 10^9/L)	13.7 (5.1)	18.0 (5.1)	<0.001	-5.4 (5.8)	-0.7 (5.1)	<0.001
Absolute neutrophil count (ANC, x 10^9/L)	5.2 (2.5)	6.6 (2.6)	<0.001	-1.3 (3.0)	0.4 (3.0)	<0.001
Platelets (x 10^9/L)	371 (166)	446 (143)	<0.001	12 (184)	28 (152)	0.50
Alanine transferase (ALT, U/L)	19 (8)	18 (8)	0.59	0.8 (11)	-0.9 (10)	0.28
Creatinine (mg/dL)	0.32 (0.11)	0.31 (0.08)	0.64	0.04 (0.13)	0.04 (0.10)	0.92

Table 4. Selected laboratory characteristics of the NOHARM randomized population. Results are shown only for participants who had data for the measure both at baseline and at 12 months, as mean (SD) or the percentage of participants affected. The median age at enrollment was 2.2 years. Treatment group differences at month 12 and changes from baseline were tested by Welch's t-tests.

FIGURE LEGENDS

Figure 1. CONSORT diagram for the NOHARM trial.

Figure 2. Incidence of malaria events in the NOHARM trial, with no statistical difference observed between the blinded treatment arms (p=0.19). Comparisons of the hydroxyurea to placebo group were calculated using Gray's test for competing events, treating death as a competing event. Solid line = hydroxyurea, dashed line = placebo. The smaller inset diagram is identical to the larger graph, but has a different scale.

Figure 3. Cumulative incidence of sickle-related adverse events over 12 months, by blinded treatment arm. Key is (A) painful vaso-occlusive crisis, p = 0.004; (B) hospitalization, p = 0.002; (C) transfusion, p = 0.27; and (D) pneumonia/acute chest syndrome, p = 0.51.

Figure 1

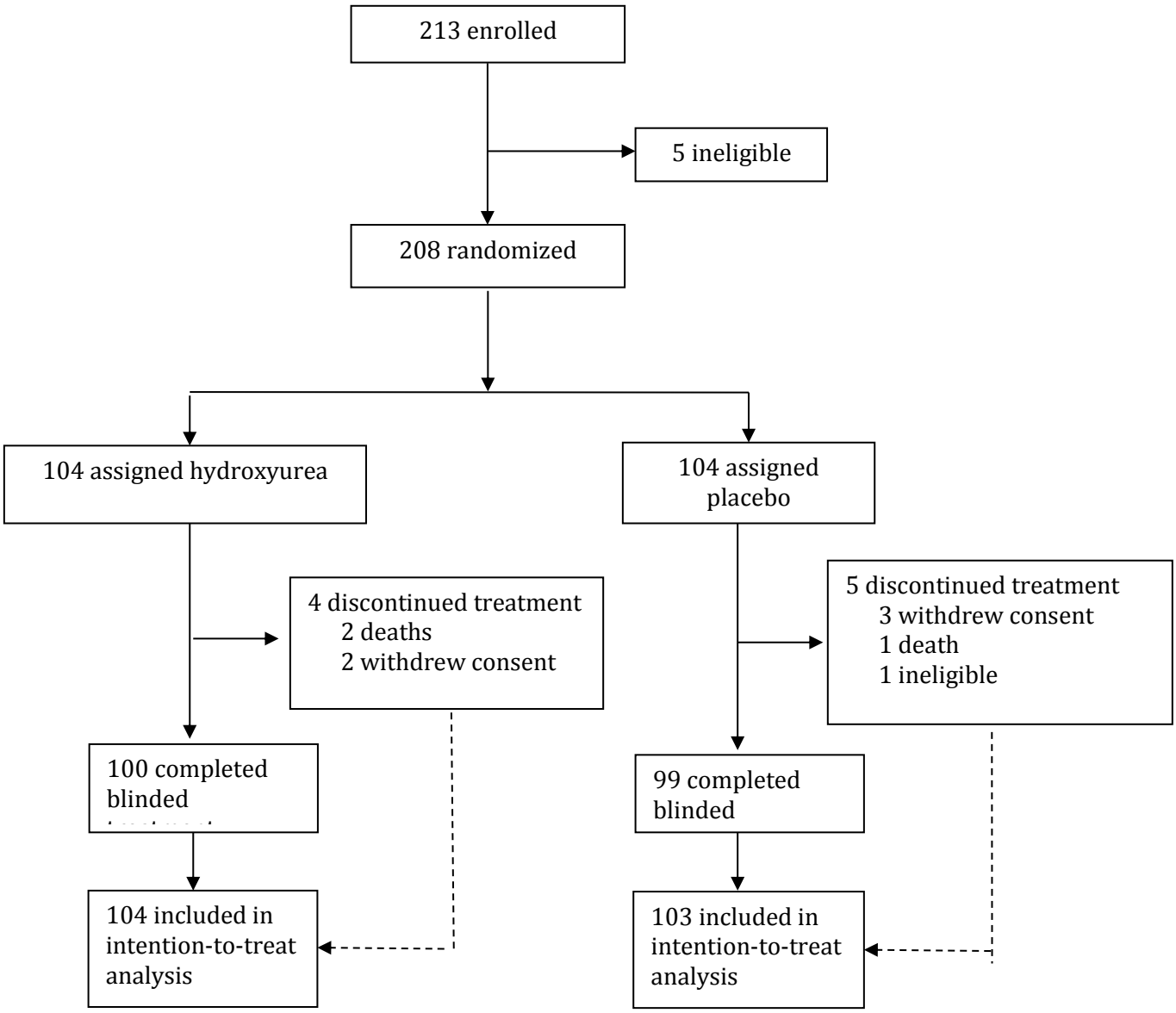
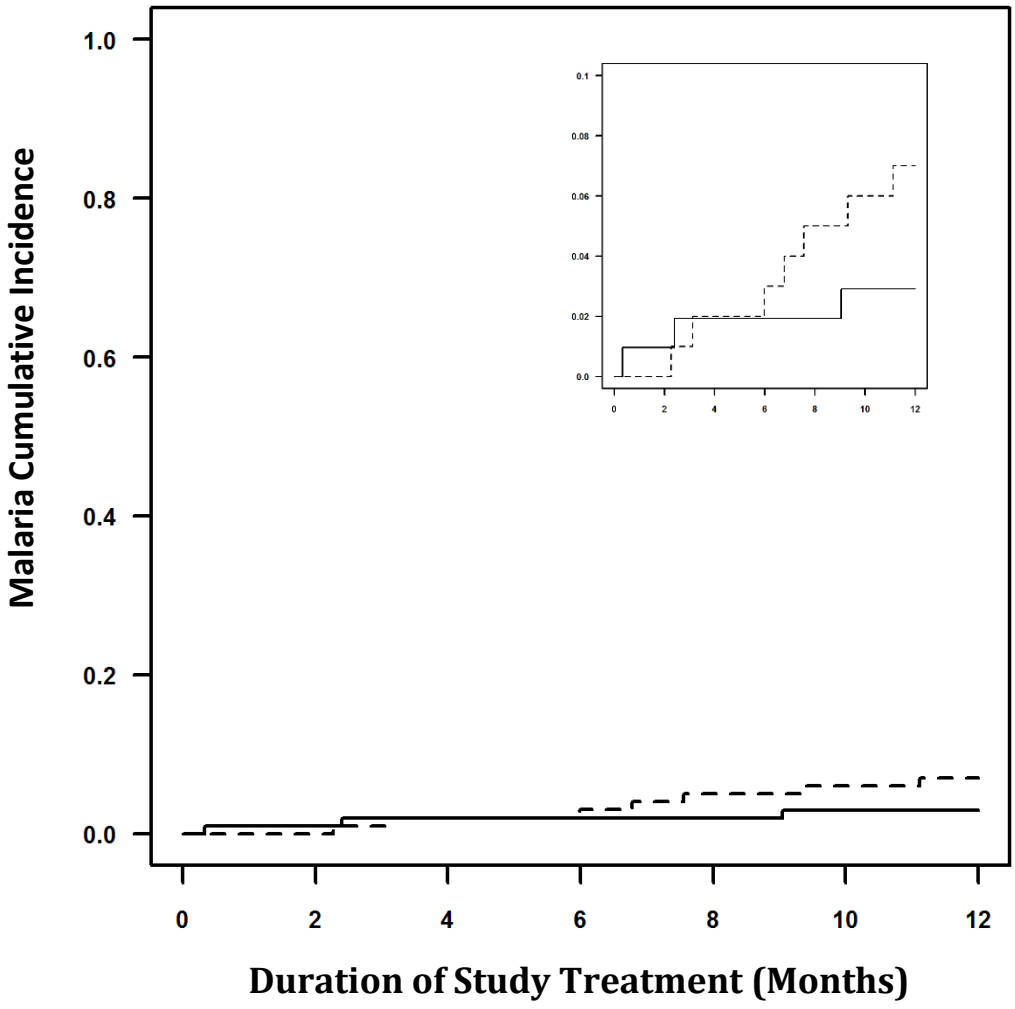
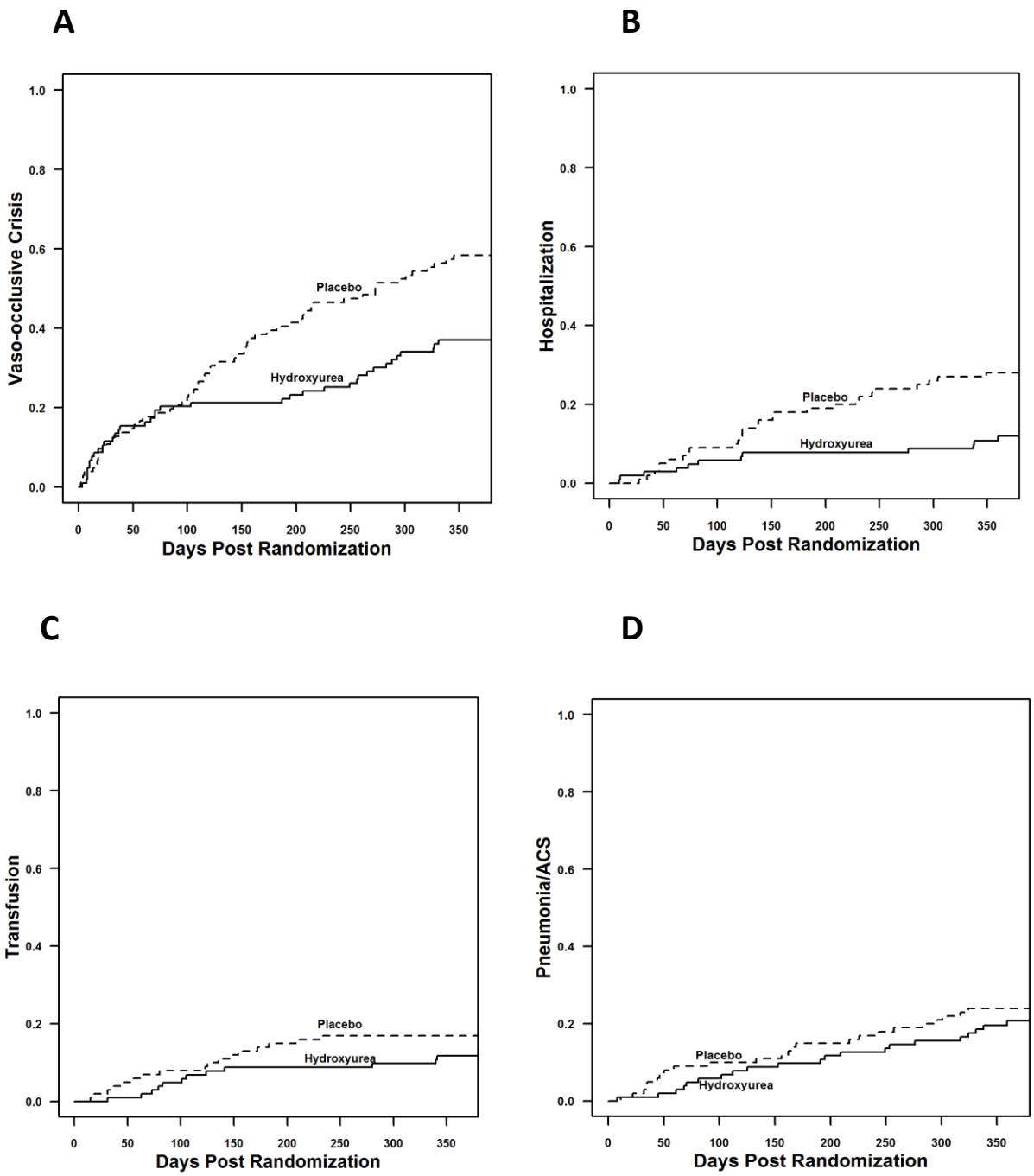

213 enrolled
5 ineligible
208 randomized
104 assigned hydroxyurea
104 assigned placebo
4 discontinued treatment
2 deaths
2 withdrew consent
5 discontinued treatment
3 withdrew consent
1 death
1 ineligible
100 completed blinded
99 completed blinded
104 included in intention-to-treat analysis
103 included in intention-to-treat analysis

Figure 2



Number at risk							
Hydroxyurea	104	102	101	99	98	97	97
Placebo	103	100	98	97	95	94	92

Figure 3


A
Vaso-occlusive Crisis
Placebo
Hydroxyurea
Days Post Randomization
B
Hospitalization
Placebo
Hydroxyurea
Days Post Randomization
C
Transfusion
Placebo
Hydroxyurea
Days Post Randomization
D
Pneumonia/ACS
Placebo
Hydroxyurea
Days Post Randomization